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High Field Asymmetric Waveform Ion Mobility Spectrometry in Nontargeted Bottom-up Proteomics of Dried Blood Spots

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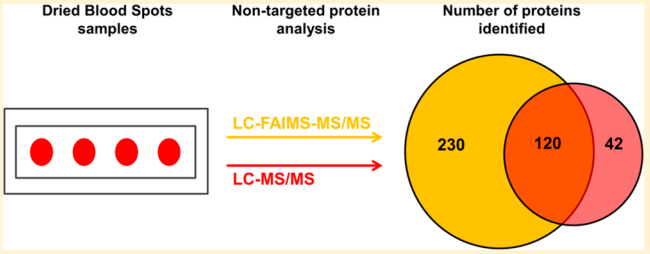
Supporting Information

ABSTRACT: Despite the great potential of dried blood spots (DBS) as a source of endogenous proteins for biomarker discovery, the literature relating to nontargeted bottom-up proteomics of DBS is sparse, primarily due to the inherent complexity and very high dynamic range associated with these samples. Here, we present proof-of-concept results in which we have coupled high field asymmetric waveform ion mobility spectrometry (FAIMS) with liquid chromatography−tandem mass spectrometry (LC−MS/MS) for nontargeted bottom-up proteomics of DBS with the aim of addressing these challenges. We, and others, have previously demonstrated the benefits of FAIMS more generally in proteomics including improved signal-to-noise and extended proteome coverage, and the aim of the current work was to extend those benefits specifically to DBS. The DBS samples were either extracted by the more traditional manual "punch and elute" approach or by an automated liquid surface extraction (LESA) approach prior to trypsin digestion. The resulting samples were analyzed by LC−MS/MS and LC−FAIMS−MS/MS analysis. The results show that the total number of proteins identified increased by ∼50% for the punch and elute samples and ∼45% for the LESA samples in the LC−FAIMS−MS/MS analysis. For both the punch and elute samples and the LESA samples, ∼30% of the total proteins identified were observed in both the LC−MS/MS and the LC−FAIMS−MS/MS data sets, illustrating the complementarity of the approaches. Overall, this work demonstrates the benefits of inclusion of FAIMS for nontargeted proteomics of DBS.



KEYWORDS: *dried blood spots, DBS, high field asymmetric waveform ion mobility spectrometry, FAIMS, differential ion mobility, proteomics, endogenous proteins*

INTRODUCTION

Proteomics-based studies are useful in the search for new biomarkers.[1] Blood is a rich source of endogenous proteins and has great potential for proteomics-based biomarker discovery;[2] however, sampling of blood from large patient cohorts can be both resource-demanding and time-consuming, making the recruitment of patients into these studies difficult. Dried blood spots (DBS) are an alternative technique for sampling of blood. This sampling technique was introduced in 1963 as a means to sampling and storage of whole blood in newborn screening.[3] DBS are easy to obtain and ship, and are well-suited to sampling from large patient cohorts, including in inaccessible regions of the world, without the need for transportation of the patient to the clinic.[4,5] Several articles have described the application of MS for targeted protein analysis of DBS,[6−12] but only two have considered nontargeted protein analysis[13−15] by use of bottom-up proteomics. This is surprising given the wide body of research on the human plasma proteome[16,17] but is likely the consequence of the complexity of blood samples and high dynamic range of protein concentrations. These challenges are not peculiar to proteomics-based studies of DBS or other blood derived samples and are typically addressed in proteomics workflows through the use of prefractionation methods such as depletion kits (for removal of high abundance proteins),[18] gel based separation techniques,[19] or strong cation exchange chromatography[20] prior to online liquid chromatography (LC)−tandem mass spectrometry (MS/MS).

The LC−MS/MS methods used in nontargeted proteomics are often several hours long, which combined with the need for sample prefractionation make discovery-based proteomics a time-consuming process. One approach by which both these issues may be addressed is the use of gas-phase separation by ion mobility spectrometry, for example, high field asymmetric waveform ion mobility spectrometry (FAIMS).[21] FAIMS has been shown to be advantageous for bottom-up proteomic analyses by improving signal-to-noise, extending proteome coverage and separating isomeric peptides.[22−29] FAIMS was introduced by Buryakov et al.[30,31] in 1991 and separates ions on the basis of their differential ion mobility in high and low electric fields. Ions are passed between two parallel electrodes to which an asymmetric waveform is applied. As a result of their differential ion mobility, the ions will become displaced from their original trajectory. This displacement can be corrected by

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superposition of a DC voltage (the compensation voltage, CV), thus allowing the ions to be transmitted through the FAIMS device. Selective transmission of ions of varying differential mobility is achieved by scanning the CV. In addition to the benefits for bottom-up proteomics, we have recently demonstrated the advantages offered by FAIMS, when coupled with liquid extraction surface analysis (LESA), for top-down protein analysis from thin tissue sections,[32,33] bacterial colonies growing on agar,[32] and DBS.[34]

Here, we have combined bottom-up proteomics of DBS with FAIMS. Two sample preparation techniques are considered: the more traditional "punch and elute" method, in which a small section of the DBS is cut out and the sample eluted into a solvent prior to tryptic digestion, and an automated surface extraction and digestion method, utilizing LESA, developed by Martin et al.[13] The samples were then analyzed by LC−MS/MS and LC−FAIMS−MS/MS. The results show a two-fold increase in the number of nonredundant proteins detected in total from both punch and elute prepared samples and LESA samples.

EXPERIMENTAL PROCEDURES

Chemicals

Formic acid (FA), ammonium bicarbonate (ABC), and acetonitrile (MeCN) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trypsin gold Mass Spectrometry grade was purchased from Promega (Madison, WI, USA).

DBS Samples

The work was approved by the University of Birmingham STEM Ethical Review Committee (ERN_14−0454). DBS were acquired from consented healthy human adult donors via finger prick onto blood spot (Guthrie) cards, Ahlstrom grade 226 filter paper from ID Biological Systems (Greenville, USA), and dried overnight.

Preparation of DBS Samples: "Punch and Elute"

Samples from DBS (n = 2) were cut out (∼5 mm diameter) and transferred to an Eppendorf vial (Eppendorf, Hamburg, Germany). Two-hundred microliters of 50 mM ABC buffer was added to the vial, and the sample was extracted on a Thermomixer from Eppendorf (800 rpm) for 2 h. A volume of 100 μL of the sample was transferred to a clean vial, and 75 μL of 0.1 μg/mL trypsin was added. The digestion was performed (vortexing, 500 rpm) at 37 °C overnight (∼18 h).

The samples were pooled and centrifuged at 14 000 rpm for 10 min prior to pipetting the supernatant into 96-well plates from Thermo Scientific (Rockford, IL, USA).

Preparation of DBS Samples: Liquid Extraction Surface Analysis (LESA)

A Triversa Nanomate from Advion Bioscience (New York, NY, USA) was used for the automatic extraction and digestion of the DBS samples. A 96-well microtiter plate from Thermo Scientific was placed in the Triversa Nanomate and 50 mM ABC buffer was added to one of the wells while 0.1 mg/mL trypsin solution was added to another well. The DBS samples were mounted (in total 10 spots) on to the 96-well plate and surface extraction and digestion of the samples was performed by using the advanced user interface (AUI) feature of the ChipSoft software. The procedure was based on the protocol published by Martin et al.[13] Seven microliters of the ABC buffer was aspirated from the well by the robotic pipet, which then relocated to a defined position over the DBS sample. Six microliters of the buffer was deposited onto the DBS surface forming a liquid microjunction. The liquid microjunction was maintained between the tip and the DBS for four seconds before 5 μL of the buffer was reaspirated. The sample was dispensed into a clean well on the 96-well plate before the tip was ejected. A new tip was selected and 4.5 μL of trypsin (0.1 mg/mL) was aspirated from the well containing trypsin and dispensed into the well containing the extracted DBS sample. The sample was then mixed by one aspiration and dispense cycle of 4 μL before the tip was ejected. The tryptic digestion was performed by incubation of the sample for 1 h at 40 °C using the temperature control unit on the Triversa Nanomate. To account for the evaporation of solvent during the digest, 7.5 μL of ABC was aspirated from the solvent well and dispensed into the sample well directly after the digest. In total, 26 surface extractions from 10 DBS samples were performed (with no resampling of previously sampled regions). In each case, individual samples were subjected to proteolysis before pooling of the resulting digests.

LC−MS

Mass spectrometry experiments were performed on a Thermo Fisher Orbitrap Elite (Bremen, Germany) equipped with a Dionex-Ultimate 3000 Nano LC system (Thermo Fisher Scientific, Sunnyvale, CA, USA). The peptides were separated using a C18 150 mm × 0.075 mm id Acclaim Pepmap 100 (pore size 100 Å, particle size 3 μm) from Thermo Scientific. All samples were desalted on a C18 5 mm × 0.30 mm id Acclaim Pepmap 100 (particle size 5 μm) trap column prior to the HPLC separation. The loading buffer, consisting of 0.1% FA, was flushed through the trap column in the first 6 min of the run. The mobile phase was run with a gradient from 3.2 to 44% MeCN in 30 min. The injection volume was 5 μL and the column temperature was 35 °C. The peptides were eluted via the Triversa Nanomate nanospray source (Advion Bioscience, New York, NY, USA) into the MS with a spray voltage of 1.7 kV. For the FAIMS analysis, the peptides were eluted into the MS via a nanoESI source equipped with a silica tip emitter (tip i.d. 10 μm) from New Objective (Woburn, MA, USA) by using a spray voltage of 3.5 kV. The Orbitrap was operated in the "top 7" data dependent analysis mode in which a survey scan (resolving power 120, 000 at m/z 400) was followed by fragmentation of the seven most abundant precursor ions within a mass range of m/z 350−1800 or 380−1600 (FAIMS). The seven most abundant ions were fragmented using collision induced dissociation (CID) with helium gas and normalized collision energy of 35%. The fragments were detected in the linear ion trap. A dynamic exclusion of 60 s was applied to prevent reselection of the precursor ion.

For the FAIMS analyses, a prototype cylindrical FAIMS device (electrode gap width of 1.5 mm) supplied by Thermo Scientific was mounted on to the Orbitrap.[35,36] The temperature of the inner electrode was set to 70 °C, while the outer electrode was set to 90 °C. The dispersion voltage (DV) was −5000 V and the CV was swept from −25 to −55 V, in 2.5 V steps, using the external CV stepping approach as described by Creese et al.[22] Nitrogen was used as the carrier gas.

Data Analysis

Data analysis was performed in Proteome Discoverer 1.4 (Thermo Fisher) using the Sequest algorithm. The MS/MS data was searched against the SwissProt human database with 20 134 sequences (reviewed and canonical, downloaded March 2017). The following parameters were applied: enzyme was set

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as trypsin; methionine oxidation was set as the variable modification; mass accuracy was set to 10 ppm for parent ion and 0.6 Da for the fragment ion (product ion type: *b* and *y*). The search also included peptides with up to two missed cleavages. The data were filtered with a strict false discovery rate (FDR) of 0.01 and a relaxed FDR of 0.05. The protein grouping filter was applied and a minimum of one high confident peptide (strict FDR 0.01) was required for a positive protein.

RESULTS AND DISCUSSION

To date, there are two publications describing nontargeted proteomics of DBS by LC−MS. In the first, Chambers et al. were able to detect 1549 peptides corresponding to 253 proteins using a labor intensive sample preparation method including 1 h elution of sample from the DBS, overnight tryptic digest, precipitation, and solid phase extraction.[14] In the second, we demonstrated the detection of 120 proteins from DBS using a simple and automatic surface extraction (liquid extraction surface analysis, LESA) followed by automated digestion of the extracted samples.[13]

Here, LC−FAIMS−MS is evaluated for the proteomic analysis of DBS samples using two different sample preparation procedures: (1) punch, elute, and overnight tryptic digest of the DBS, referred to here as punch and elute, and (2) liquid extraction surface analysis (LESA) of DBS combined with a 1 h tryptic digest using the Triversa Nanomate robotic platform from Advion as described by Martin et al.[13] Both sample preparation procedures were relatively efficient in that no cleanup of the samples was performed; however, the LESA procedure was faster than the punch and elute procedure (2 h elution and ∼18 h digest) as only four seconds surface extraction and 1 h tryptic digest of the sample was required.

The samples from the punch and elute procedure were pooled after digestion, as were those from the LESA procedure, to ensure that any differences observed were due to the FAIMS and not differences in extraction or digestion of the proteins. Eight replicates of the LC−MS/MS analysis without FAIMS (described hereafter as without FAIMS) were performed to evaluate the effect of reanalysis on the total number of peptides and corresponding proteins identified. For the samples run with LC−FAIMS−MS/MS (described hereafter as FAIMS or with FAIMS), a prototype cylindrical FAIMS device was used.[35,36] External CV stepping (i.e., multiple LC−MS/MS analyses each at constant, and different, CV) was chosen rather than the internal CV stepping (scanning of CV values within one analytical run) as this has previously been shown to result in greater numbers of peptide identifications.[22] The CV range was −55 to −25 V with 2.5 V steps (13 LC−FAIMS−MS/MS analyses in total) and was chosen based on previous optimization of CV range for peptides.[23,37,38]

Numbers of Detected Peptides and Proteins

The average and total number of nonredundant proteins and peptides detected with and without FAIMS are shown in Table 1. The list of the proteins detected is shown in Supplementary Table S1 (for punch and elute without FAIMS), Supplementary Table S2 (for punch and elute with FAIMS), Supplementary Table S3 (for LESA without FAIMS), and Supplementary Table S4 (for LESA with FAIMS). All proteomics data, together with annotated MS/MS spectra for single peptide protein identifications, are available via ProteomeXchange with identifier: PXD007926.

Table 1. Average Number of Unique Peptides and Proteins and Total Number of Nonredundant Peptides and Proteins Detected with and without FAIMS from DBS Using Punch and Elute Procedure and LESA Procedure

experiment	peptides[a]	proteins[a]	nonredundant peptides	nonredundant proteins
A: Punch and Elute				
without FAIMS	191	68	536	162
with FAIMS	124	80	1065	350
B: LESA				
without FAIMS	288	81	784	187
with FAIMS	119	68	1077	272

[a]Average number.

Punch and Elute

For the punch and elute samples, a total of 536 nonredundant peptides, corresponding to 162 proteins, were identified in the analyses without FAIMS (Table 1A). On average, 191 peptides, corresponding to 68 proteins, were identified per LC−MS/MS run. The effect of reanalysis (without FAIMS) of the punch and elute samples can be seen in Figure S1 in the Supporting Information. In the first run of the punch and elute samples, 260 peptides were detected. The total number of peptides detected increased to 527 over the next five LC−MS analyses of the sample. There was no remarkable increase in the overall number of peptides detected after six analyses.

For the punch and elute samples analyzed with FAIMS, the total number of nonredundant peptides was 1065, corresponding to 350 proteins. The average number of peptides identified per CV step was 124, corresponding to 80 proteins (Table 1A). Figure 1A shows the number of detected peptides and proteins for each CV step. The fewest peptides identified in a single CV step was 38 (CV = −55 V), and the greatest number was 210 (CV = −37.5 V). The results show that incorporation of FAIMS into the workflow results in a two-fold increase in the numbers of peptides and proteins identified from the punch and elute samples.

The results obtained for the punch and elute samples with FAIMS compare favorably (increase of 38% in protein detection) with those obtained by Chambers et al.[14] Chambers et al. were able to detect 1549 peptides corresponding to 253 proteins from DBS using three replicates. The total instrument time in those experiments was approximately 5 h, and sample preparation was labor intensive including SPE and overnight lyophilization after the overnight tryptic digest. In our experiments, the total instrument time was 13 h when using FAIMS with different CV steps; however, sample preparation was minimal. A more refined sample preparation procedure of the DBS may further increase the number of detected proteins found when using LC−FAIMS−MS/MS.

LESA

For the LESA samples without FAIMS, a total of 784 nonredundant peptides, corresponding to 187 proteins, were identified from eight repeat LC−MS/MS analyses (Table 1B). The average number of unique peptides detected was 288, corresponding to 81 proteins. These results are comparable with those obtained by Martin et al.[13] who were able to detect 114 proteins on average (three replicates) by using LESA extraction of DBS samples followed by tryptic digest and LC−MS/MS. The increased number of peptides from the LESA samples (without FAIMS) compared to punch and elute

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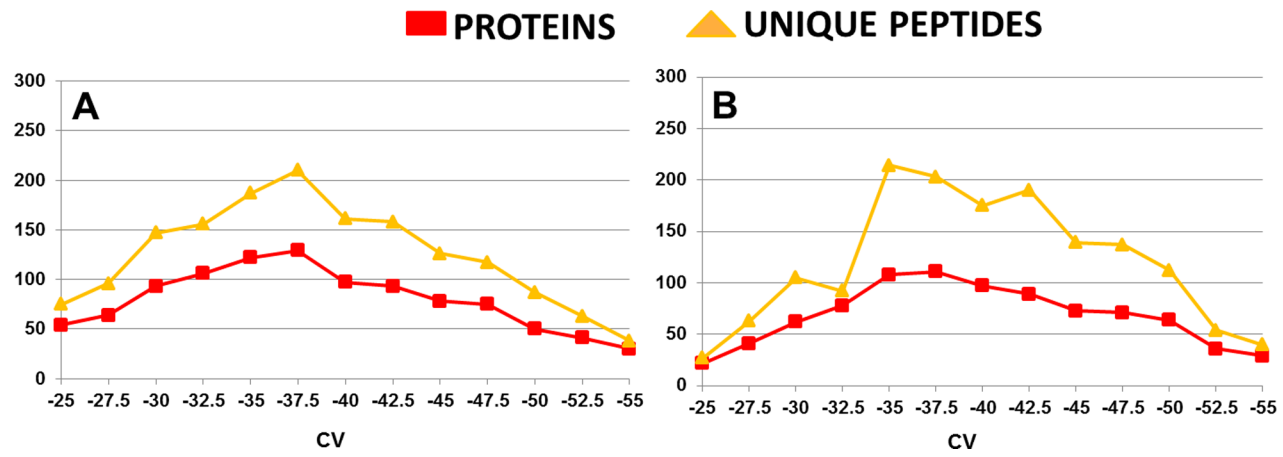

Figure 1. Number of detected proteins (red) and peptides (yellow) for each of the CV steps in the LC−FAIMS−MS/MS analyses of (A) punch and elute samples and (B) for LESA samples.

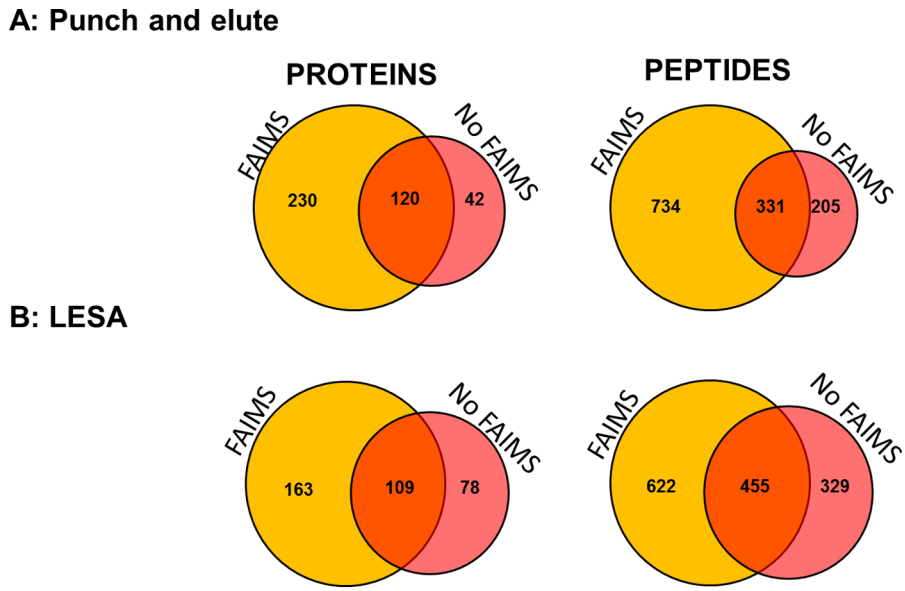

Figure 2. Venn diagrams illustrating total nonredundant proteins and peptides identified when analyzing the samples with FAIMS (all 13 CV steps) and without FAIMS (eight analysis replicates) for (A) punch and elute samples and (B) LESA extracted samples.

samples (without FAIMS) was mostly due to an increased number of peptides from high abundance proteins (e.g., serum albumin, complement 3 and alpha-2-macroglobulin). As observed for the punch and elute samples, the total number of detected peptides increased from 476 in the first run to 744 after five additional LC−MS analyses of the LESA samples (Supplemental Figure S1B). No significant increase was observed after six runs (as also observed for the punch and elute sample).

A total of 1077 nonredundant peptides were identified, corresponding to 272 proteins, from the LESA samples analyzed with FAIMS. That corresponds to an increase of 37% in peptide identifications and 45% in protein identifications over the LESA samples analyzed without FAIMS. The average number of peptides identified per CV step was 119, and as can be seen in Figure 1B, the fewest peptides were identified at CV −25 V (27 peptides) and the greatest number at CV −35 V (214 peptides).

The results obtained for the LESA samples with FAIMS are an improvement over those obtained by Martin et al.[13] without FAIMS (by over 2-fold) and are comparable with those obtained by Chambers et al.[14] using the punch and elute extraction method, but with reduced sample preparation requirements.

Comparison with Plasma Proteomics

It is useful to consider our results with those obtained in plasma proteomics experiments. For example, Keshishian et al.[39] combined immunodepletion of proteins of high- and moderate abundance with prefractionation of peptide digests (for a total of 30 peptide fractions) and long liquid chromatography gradients (3 h analyses) to identify >5000 proteins in human plasma samples. This result is undoubtedly impressive, but comes with the caveat that, in addition to the extensive sample preparation, the total MS analysis time was ∼90 h. More recently, Mann and co-workers developed a workflow for plasma proteomics in which ∼5 μL of blood is centrifuged and ∼1 μL of plasma harvested.[40] The plasma is subsequently digested with trypsin for 1 h and the resulting peptides analyzed by LC−MS/MS using a 20 min LC gradient. The total time for this workflow was ∼3 h. The average number of proteins identified per individual in the absence of peptide prefractio-

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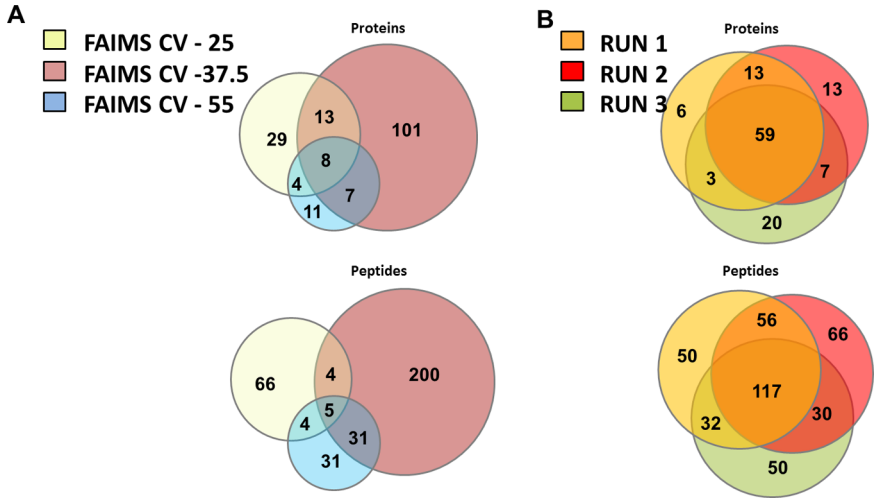

Figure 3. Redundancies of proteins found when analyzing the punch and elute samples with (A) FAIMS using three different CV steps and (B) three analysis replicates without FAIMS.

nation was around 200, rising to ~300 when a matching library algorithm was employed. That is, the numbers of proteins identified were similar to those identified in our DBS experiments when FAIMS was employed.

LESA versus Punch and Elute

The numbers of proteins identified following LESA and punch and elute are similar when the DBS samples were analyzed without FAIMS. The number of identified proteins was higher for the punch and elute samples (29% more proteins identified) when the samples were analyzed with FAIMS. Further experiments are required to determine the significance of these observations, but we predict that more proteins are transferred from the sample and into the buffer for the punch and elute approach (2 h elution) than for the LESA approach (four seconds surface extraction). In the absence of FAIMS, the increased number of proteins in the punch and elute samples will probably be dominated by peptides from the highly abundant proteins. FAIMS filters out many peptides from highly abundant proteins, thus enabling the detection of peptides in lower concentrations. One example is serum albumin, which was represented with around 13 unique peptides in samples run without FAIMS (punch and elute). In samples run with FAIMS (punch and elute), this protein was represented with six peptides at CV −32.5 V, and only with one peptide when using CV −37.5 V. Another example is the protein complement C3 which was represented by 32 unique peptides in the analysis without FAIMS (LESA), but with only seven unique peptides in the analysis with FAIMS (CV −40 V). Supplementary Table S5 shows some of the highly abundant proteins and the numbers of unique peptides detected both from punch and elute samples and LESA samples analyzed with and without FAIMS. The table shows that more peptides from high abundance proteins were detected from samples analyzed without FAIMS and may explain the increased overall number of proteins identified in samples analyzed with FAIMS.

Complementarity of Analyses with and without FAIMS

The complementarity of the results obtained following analyses performed with and without FAIMS is illustrated in Figure 2. Figure 2A shows Venn diagrams of the total number of nonredundant peptides and proteins identified from the punch and elute samples analyzed with and without FAIMS. The total number of proteins identified by both approaches was 392, with 59% unique to FAIMS, 11% unique to non-FAIMS, and 30% (120 proteins) observed in both. The total number of peptides identified was 1270, with 58% unique to FAIMS, 16% unique to non-FAIMS, and 26% (331 peptides) common to both. A similar trend was observed for the LESA samples (Figure 2B). A total of 350 proteins were identified, with 47% unique to FAIMS, 22% unique to non-FAIMS, and 31% (109 proteins) observed in both. For peptides the total number was 1406, with 44% unique to FAIMS, 23% unique to non-FAIMS, and 32% (455 peptides) seen in both. These results are in agreement with earlier work demonstrating the complementarity of LC−MS/MS workflows incorporating FAIMS and without FAIMS in terms of peptide and protein identification.[22,23]

Redundancy between CV Steps

Figure 3A shows a Venn diagram of proteins identified in the punch and elute samples analyzed at CV steps −25, −37.5, and −55 V. Approximately 60% (33 proteins) of proteins identified at CV = −25 V were not identified when the same sample was run with CV = −37.5 V, and around 50% (15 proteins) of the proteins identified at CV = −55 V were not found when using CV = −37.5 V. The number of proteins identified in all three analyses was eight, corresponding to less than 5% of the total. Figure 3B shows a Venn diagram of proteins found in three LC−MS/MS analyses performed in the absence of FAIMS. 49% of the peptides identified in these analyses were observed in all three runs, and 68% were identified in two or more analyses. A similar analysis was performed for the LESA samples, see Supporting Information Figure S2. For the FAIMS analyses, 7% of the total proteins identified were observed at all three CV steps (CV = −25, −37.5, and −55 V). For the non-FAIMS analyses, 53% of proteins were identified in all three analyses and 65% were identified in two or more analyses. Total ion chromatograms from samples run with FAIMS (CV = −25, −37.5 and −55 V) and without FAIMS are shown in Figure S3 (punch and elute samples) and Figure S4 (LESA samples) in the Supporting Information. These chromatograms further confirm that different information is acquired with and without FAIMS.

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Table 2. Lower Abundance Proteins Found in DBS Samples Prepared with Punch and Elute Approach and LESA Approach and Analyzed with or without FAIMS

		punch and elute		LESA	
proteins	concentration[a]	no FAIMS	FAIMS (CV (V))	no FAIMS	FAIMS (CV (V))
apolipoprotein L1	26−88[b]	no	−37.5, −40	no	−35, −37.5
protein S100 A8	05−1.5[b]	no	no	no	−35, −37.5
protein S100 A9	0.7−1.9[b]	no	−30, −32.5, −37.5	yes	−30, −35, −42.5
glyceraldehyde-3-phosphate dehydrogenase	0.091[c]	yes	−30 to −50	yes	−27.5 to −37.5, −47.5
vinculin	0.090[c]	no	−32.5, −42.5, −47.5	no	−45
carbonic anhydrase 2	0.042[c]	yes	−32.5, −35, −42.5, −47.5, −50	yes	−30 to −50
beta-parvin	0.0031[c]	no	−35, −42.5	no	no
prothrombin	0.0012[c]	no	−47.5, −50	no	−37.5, −42.5, −45, −47.5

[a]Measured in μg/mL. [b]From ref 41. [c]From ref 42.

Charge State Distributions

The charge states of the peptides detected for samples analyzed with and without FAIMS were evaluated and can be seen in Figure S5 in the Supporting Information. Without FAIMS, the percentage of peptides with charge states ≥ +3 charges were fairly constant at ∼30% from run to run. When the samples were analyzed with FAIMS, an increase in the percentage of peptides with charge states ≥ +3 was observed as the CV voltage was lowered (more negative). The same trend was also observed for the punch and elute samples (data not shown). This distribution of peptide m/z with varying CV has been described previously.[23,38]

Detection of Lower Abundance Proteins

Table 2 shows examples of proteins of lower abundance (as described by Hortin et al.[41] and Liu et al.[42]) that were identified from the DBS samples and the typical plasma concentration of these proteins. As discussed above, many high abundance proteins were detected in samples analyzed both without FAIMS and with FAIMS; however, fewer signature peptides from high abundance proteins were seen with FAIMS. Proteins that are at the lower concentration range of the typical high abundance proteins (<100 μg/mL) were also detected: apolipoprotein L1, protein S100-A8 and A9. Proteins with plasma concentrations less than 100 ng/mL were also detected: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), carbonic anhydrase 2, vinculin, beta-parvin, and prothrombin. For the punch and elute samples, only GAPDH and carbonic anhydrase 2 were observed in the non-FAIMS analyses, and all but S100 A8 were observed in the FAIMS analyses. For the LESA samples, S100 A9, GAPDH, and carbonic anhydrase were observed without FAIMS, and all but beta-parvin were observed with FAIMS.

Although S100 A8, apolipoprotein L1, and prothrombin were only observed here with FAIMS analysis, it should be noted that both were observed previously by Martin et al.[13] (LESA, no FAIMS) and Chambers et al.[14] (punch and elute, no FAIMS). Similarly, S100 A9 was not observed here in the punch and elute samples with FAIMS but was seen previously by Chambers et al. Glyceraldehyde-3-phosphate dehydrogenase and carbonic anhydrase 2 were seen both without and with FAIMS and were also observed by both Chambers et al. and Martin et al. Vinculin and beta-parvin were not detected in the samples from either Chambers et al. or Martin et al.

CONCLUSIONS

Here, we have evaluated FAIMS coupled with LC−MS/MS for nontargeted proteomics of DBS and have considered two DBS sample preparation methods, the well-established punch and elute approach and the more recent LESA approach. These proof-of-concept results show that for both sample preparation approaches, the inclusion of FAIMS in the LC−MS/MS workflow results in an approximately two-fold increase in the number of proteins identified. Our findings also show the complementarity of data sets obtained with and without FAIMS. Between one-quarter and one-third of the total proteins identified were common to both FAIMS and non-FAIMS data sets, with over 40% unique to FAIMS. Overall, our results suggest that FAIMS may have a role to play in DBS proteomics by addressing the challenges of sample complexity and dynamic range.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jproteome.7b00746.

Total number of detected peptides from LC−MS/MS reanalysis of punch and elute and LESA samples; redundancies of proteins found when analyzing LESA samples with FAIMS and without FAIMS; total ion chromatograms obtained for punch and elute samples analyzed with and without FAIMS; total ion chromatograms obtained for LESA samples analyzed with and without FAIMS; percentage of peptides with +2 charges and ≥ + 3 charges for LESA samples analyzed without and with FAIMS using different CV steps (PDF)

Punch and elute samples analyzed without FAIMS (XLSX)

Punch and elute samples analyzed with FAIMS (XLSX)

LESA samples analyzed without FAIMS (XLSX)

LESA samples analyzed with FAIMS (XLSX)

Unique peptides detected for some high abundance proteins from punch and elute and LESA samples with and without FAIMS (XLSX)

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Notes

The authors declare no competing financial interest.
Supplementary data supporting this research is openly available from the University of Birmingham data archive at (http://findit.bham.ac.uk). The MS proteomics data have been deposited in the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE[40] partner repository with the dataset identifier PXD007926.

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